

Original Research Article**PHYTOCONSTITUENTS AND EVALUATION OF ACETYLCHOLINESTERASE INHIBITION BY METHANOL EXTRACT OF *LIQUIDAMBAR STYRACIFLUA* (L.) AERIAL PARTS**

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ABSTRACT

This study deals with evaluation of acetylcholinesterase activity of *Liquidambar styraciflua* aerial parts methanol 80% extract and identification of the bio-active phytoconstituents of the extract. The acetylcholinesterase inhibition was detected by Ellman's method and the methanol extract was subjected for phytochemical analysis. The extract of *L. styraciflua* has shown ($IC_{50} = 0.070$ mg/mL), with a similar activity to neostigmine ($IC_{50} = 1.87$ μ g/mL) and galanthamine ($IC_{50} = 0.37 \times 10^{-3}$ mg/mL) which are the most effective compounds in the treatment of Alzheimer's disease. Phytochemical investigation of methanol extract of *L. styraciflua* revealed the presence of triterpenes, flavonoids, tannins and carbohydrates and chromatographic separation and fractionation of methanol extract of *L. styraciflua* resulted in the isolation of β -sitosterol, lupeol, oleanolic acid, ursolic acid, luteolin, orientin, isoorientin, kaempferol 3-O- α -rhamnoside and kaempferol 3-O- β -glucoside. These results suggest that methanol extract of *L. styraciflua* is a good natural source for acetylcholinesterase inhibitor.

Keywords: *Liquidambar styraciflua*, aerial parts, Anticholinesterase activity, Alzheimer's disease, phytoconstituents

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INTRODUCTION

Alzheimer's disease (AD) is one of the most widespread neurodegenerative diseases that involve dementia and mainly afflict people over 65 years of age. The therapy of early and moderate stages of AD is mainly based on acetylcholine esterase inhibitors such as synthetic donepezil and galanthamine. However, these licensed medicines have drawbacks of inducing severe peripheral and central side effects, including gastrointestinal disturbances, insomnia, fatigue or depression. On the other hand, since the butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) activity in AD increases progressively as the severity of dementia progresses, researchers have investigated selective BuChE and AChE inhibitors in the treatment of AD as well [1]. The serious side effects caused by licensed drugs used to treat AD have forced researchers to investigate safer AChE inhibitors from natural sources. One of the best sources of new substances to treat AD are natural products and their derivatives. Traditionally, plants have been used to enhance memory and to alleviate other symptoms associated with AD [2]. *Liquidambar styraciflua* (L.) from Hamamelidaceae family, is known as sweet gum tree. It is native to southeastern, east-central, and south-central United States, southern Mexico and central America [3]. The bark is grayish brown, deeply furrowed into narrow, somewhat rounded ridges. The leaves are alternate, simple, dark green and lustrous

above. Flowers are monoecious consisting of 2-beaker ovaries subtended by minute scales. The fruit is a dangling brown, woody spiny tipped "gum ball" with seeds brownish and winged [4, 5]. In traditional medicine, *L. styraciflua* is used in treatment of wounds, the roots were boiled into a strong tea to treat skin sores, diarrhea and dysentery [6]. The bark was used to make an infusion that was used as a sedative for nervous patients and for patients who were well in the day but sick during the night. The plant was used to treat colic, internal diseases and to "comfort the heart." [6]. One report about cancer chemopreventive activity from triterpenoids from the cones of the plant [7]. The main aim of this present study is to evaluate acetylcholinesterase inhibitory activity of *L. styraciflua* aerial parts methanol extract and also investigate phytochemical content of the plant extract.

MATERIALS AND METHODS

General experimental procedures

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt). ^1H -NMR and ^{13}C -NMR (Varian Unity Inova). MS (Finnigan MAT SSQ 7000, 70 ev). (Silica gel (0.063-0.200 mm for column chromatography) and Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) F₂₅₄ plates. Solvent mixtures, BAW (*n*-butanol: acetic acid: water 4:1:5 upper phase, 15% acetic acid: water: glacial acetic acid: 85:15). Paper Chromatography (PC) Whatman No.1 (Whatman Led.Maid Stone, Kent, England) sheets for qualitative detection of flavonoids and sugars were used in this study.

Plant identification and collection

Liquidambar styraciflua aerial parts were collected from Al-Zohiriya garden, Giza, Egypt in May 2011. The plant was identified by Dr. Mohammed El-Gebaly, department of botany, national research centre (NRC) and by Mrs. Tereeza Labib consultant of plant taxonomy at the ministry of agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen was deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

Plant extract preparation

Air dried aerial parts of *L. styraciflua* (760 g) were extracted with methanol: distilled water 80:20 (v/v) several times at room temperature by maceration method. The extract was concentrated under reduced pressure to give 37 g of methanol extract. The extract was phytochemically screened according to that described by Yadav and Agarwala [8].

Isolation of the bioactive components of methanol extract of *L. styraciflua*

Liquidambar styraciflua (35 g) of aerial parts methanol extract was subjected to silica gel column chromatography eluting with hexane, dichloromethane, ethyl acetate and methanol gradually.

One hundred and fifty fractions of 100 ml conical flask were collected. The fractions that showed similar Paper Chromatography (PC) in two solvent systems, butanol–acetic acid–water (BAW) 4:1:5 and 15% acetic acid were combined to give 4 fractions (I, II, III and IV). Fraction I (2.1 g) was subjected to sub-column of silica gel eluted with *n*-hexane: dichloromethane (50:50) gave compound 1 and elution with dichloromethane: *n*-hexane (80:20) gave compound 2.

Fraction II (1.45 mg) was subjected to sub-column of silica gel eluted with dichloromethane: ethyl acetate (90:10) yielded compound 3 and elution with dichloromethane: ethyl acetate (95:5) gave compound 4. Fraction III (1.85 g) was subjected to sub-column of silica gel eluted with ethyl acetate : methanol (95: 5) to give compound 5 while compound 6 was obtained from elution with ethyl acetate: methanol (90:10) and

also compound 7 was obtained by elution with ethyl acetate: methanol (80:20). Fraction IV (1.25 g) was subjected to sub-column of silica gel eluted with ethyl acetate: methanol (70:30) gave compound 8 while compound 9 was obtained by elution with ethyl acetate: methanol (50:50). All the isolated compounds were purified on sephadex LH-20 column using different systems of methanol and distilled water.

General method for acid hydrolysis of flavonoid glycosides

5 mg of each flavonoid glycoside 6, 7, 8 and 9 in 5 ml 10% HCl was heated for 5 h. The aglycones were extracted with ethyl acetate and identified by co-TLC with authentic standards. The sugars in the aqueous layer were identified by co-paper chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in solvent system (*n*-BuOH-AcOH-H₂O 4:1:5 upper layer).

Acetylcholinesterase inhibition assay

The methanol extract of *L. styraciflua* was dissolved in methanol to prepare solution of 10 mg/mL. Then, 1.5 µL of the extract was spotted on silica gel TLC plate and developed with chloroform: methanol 9:1 after which the enzyme inhibitory activity was detected using Ellman's method "in situ" on the plate [9, 10]. The developed plate was sprayed with 1 mM Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid)] (DTNB) and 1 mM acetylthiocholine iodide (ATCI) in buffer A. It dried for 3-5 minutes, then an enzyme solution of AChE from an electric eel (type VI-s lyophilized, 261 U/mg solid, 386 U/mg protein) dissolved in buffer A (500 U/mL stock solution) was diluted with buffer A to obtain 5 U/mL enzyme and was then sprayed on the plate [10]. Yellow background with white spot for inhibiting extract was visible after about 5 minutes. The observation must be recorded within 15 minutes because they fade after 20-30 minutes. To observe whether the positive results of the extract in TLC or the microplate assay are due to enzyme inhibition or to the inhibition of the chemical reaction between DTNB and thiocholine, (the product of the enzyme reaction), 5 units/mL of AChE was premixed with 1 mM ATCI in buffer A and incubated for 15 minutes at 37°C. This enzyme-substrate mixture was used as thiocholine spray [10]. The extract was spotted on the silica gel TLC plate developed as described above and sprayed with 1 mM solution DTNB followed by the thiocholine spray. White spot on a yellow background was observed for false positive extract.

The inhibitory effect quantitative of methanol extract of *L. styraciflua* on acetylcholinesterase activity is evaluated using and adaptation of the spectrophotometric method of Ellman et al. [9] modified by Rhee et al. [10]. Five different concentrations were prepared in triplicate, starting from the methanol extract of *L. styraciflua* (1 mg/mL; 0.5 mg/mL; 0.25 mg/mL; 0.125 mg/mL and 0.0625 mg/mL). The reaction was monitored at 412 nm for 5 min in spectrophotometer.

In test tube is placed 100 µL of the extract (concentration 0.1% solution in 50 mM Tris-HCl pH 8, and methanol 10%) was mixed with 100 µL of AChE 0.22 U / ml (22 U of enzyme diluted in 100 mL of 50 mM Tris-HCl pH 8, 0.1% BSA) and 200 µL of buffer (50 mM Tris-HCl, pH 8, BSA 0.1%). Incubating the mixture for 5 min at 30°C. Subsequently add, 500 µL of DTNB (concentration of the 3 mM in Tris-HCl pH 8, 0.1 M NaCl, 0.02 M MgCl₂) and 100 µL of ATCI (4 mM in water). A blank should also be prepared by substituting AChE with 100 µL of buffer (50 mM Tris-HCl buffer pH 8, 0.1% BSA). The reaction is monitored for 5 min at 412 nm and initial velocity (V₀) recorded. Anticholinesterase activity (%) was calculated:

$$I(\%) = (1 - V_{0 \text{ sample}}) \times 100 / V_{0 \text{ white}}$$

Sample V₀ and V₀ represents the initial rates blank samples and white.

Inhibition concentration 50% (IC₅₀) values so obtained by plotting Log-Probbit. Neostigmine (or other commercial acetylcholinesterase inhibitor) is used as positive control at the same.

RESULTS

The present study was focused on the evaluation of acetylcholinesterase activity of *L. styraciflua* aerial parts methanol extract and the extract showed a significant inhibition for acetylcholinesterase. enzyme with ($IC_{50} = 0.070$ mg/mL). We investigated the presence of phytochemicals and bioactive constituents in *L. styraciflua* methanol extract. Phytoconstituents are shown in table 1. The major bioactive components of *L. styraciflua* aerial parts methanol extract are β -sitosterol, lupeol, oleanolic acid, ursolic acid, luteolin, luteolin 8-C- β -glucoside (orientin), luteolin 6-C- β -glucoside (isoorientin), kaempferol 3-O- α -rhamnoside and kaempferol 3-O- β -glucoside. The chemical structures of the bio-active components were elucidated by different spectroscopic analyses and shown in Figure 1.

Table 1. Phytochemical analysis of the methanol extract of *L. styraciflua* aerial parts

Chemical Constituents	Methanol extract
Carbohydrates and/or glycosides	+
Tannins	
a. Condensed tannins	+
b. Hydrolysable tannins	+
Alkaloids and/or nitrogenous bases	-
Flavonoids	+
Sterols and/or triterpenes	+
Saponins	-
Coumarins	-
(+) indicate the presence of constituents, (-) indicate the absence of constituents	

Structure elucidation of the isolated compounds

Compound 1 (β -sitosterol): 20 mg, white needles, 1H -NMR (400 MHz, $CDCl_3$): δ 5.37 (1H, m, H-6), 3.52 (1H, m, H-3), 1.09 (3H, s, CH_3 -19), 0.98 (3H, d, $J = 6.5$, CH_3 -21), 0.92 (3H, t, $J = 7.4$, CH_3 -29), 0.85 (3H, d, $J = 6.7$ Hz, CH_3 -26), 0.81 (3H, d, $J = 6.7$ Hz, CH_3 -27), 0.75 (3H, s, CH_3 -18). ^{13}C -NMR (100 MHz, $CDCl_3$): δ 140.4 (C-5), 121.5 (C-6), 71.6 (C-3), 57.2 (C-17), 56.4 (C-14), 50.3 (C-9), 46.3 (C-24), 42.8 (C-13, 4), 39.8 (C-12), 37.6 (C-1), 36.7 (C-10), 35.9 (C-20), 34.2 (C-22), 31.7 (C-8, 7), 31.4 (C-2), 29.2 (C-25), 28.4 (C-16), 26.2 (C-23), 24.5 (C-15), 23.4 (C-28), 21.1 (C-11), 19.8 (C-26), 19.5 (C-19), 19.2 (C-27), 18.6 (C-21).

Compound 2 (Lupeol): 12 mg, white powder, 1H -NMR ($CDCl_3$, 400 M Hz): δ 0.75, 0.8, 0.85, 0.96, 0.98, 1.08, 1.75 (each 3H, s), 3.25 (1H, dd, $J = 5.6, 10.8$ Hz, H-3), 4.58 (1H, s, H-29a), 4.68 (1H, s, H-29b). ^{13}C -NMR($CDCl_3$, 100MHz): δ 151.4 (C-20), 108.7 (C-29), 78.6(C-3), 55.8 (C-5), 50.7 (C-9), 48.7 (C-18), 48.4 (C-19), 43.2 (C-17), 43.2 (C-14), 40.8 (C-8), 39.7 (C-22), 38.7 (C-4), 38.5 (C-1), 38.7 (C-13), 37.6 (C- 10), 35.7 (C-16), 34.5 (C-7), 29.4 (C-21), 28.4 (C-23), 27.6 (C-2), 27.6 (C-15), 25.4 (C-12), 21.4 (C-11), 19.4 (C-30), 18.7 (C-6), 18.4 (C-28), 16.5(C-25), 16.2 (C-26), 15.7(C-24), 15.2 (C-27).

Compound 3 (Oleanolic acid): 9 mg, white amorphous powder. 1H -NMR ($CDCl_3$, 400 MHz): δ 5.23 (1H, t, $J=3.4$, H-12), 3.17 (1H, dd, $J=10, 4.2$ Hz, H-3), 2.74 (1H, dd, $J=12.5, 4$ Hz, H-18), 0.95 (3H, s, Me-23), 0.76 (3H, s, Me-24), 0.85 (3H, s, Me-25), 0.77 (3H, s, Me-26), 1.23 (3H, s, Me-27), 0.89 (3H, s, Me-29), 0.95 (3H, s, Me-30). (+) ESI-MS: m/z 455 $[M-H]^+$.

Compound 4 (Ursolic acid): 10 mg, White powder. 1H -NMR ($CDCl_3$, 400 MHz): δ 5.26 (1H, t, $J=3.5$, H-12), 3.17 (1H, dd, $J=10, 4.2$ Hz, H-3), 2.15 (1H, d, $J=11.5$ Hz, H-18), 1.92 (1H, dd, $J=12.8, 4.2$ Hz, H_b-22), 1.12

(1H, m, H_a-22), 1.22 (3H, s, Me-23), 0.94 (3H, s, Me-24), 0.75 (3H, s, Me-25), 1.04 (3H, s, Me-26), 1.12 (3H, s, Me-27), 0.92 (3H, d, *J* = 6.4 Hz, Me-29), 0.89 (3H, d, *J* = 5.8 Hz, Me-30). (+) ESI-MS: *m/z* 455 [M-H]⁺.

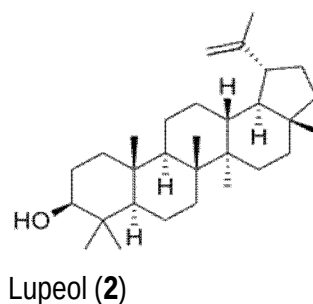
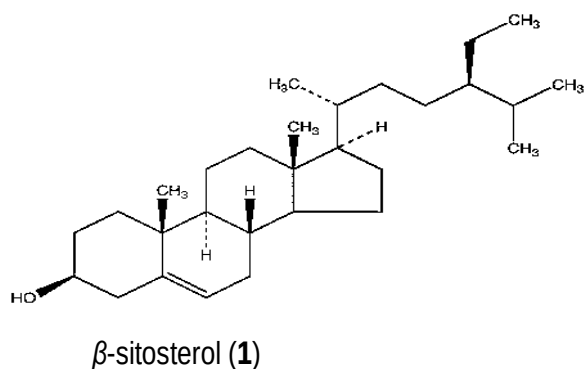
Compound 5 (Luteolin): 11 mg, yellow powder, ¹H-NMR: δ ppm 12.9 (1H, s, 5-OH), 7.4 (1H, d, *J* = 8 Hz, H-6'), 7.38 (1H, d, *J* = 2 Hz, H-2'), 6.85 (1H, d, *J* = 8 Hz, H-5'), 6.6 (1H, s, H-3), 6.4 (1H, d, *J* = 2 Hz, H-8), 6.15 (1H, d, *J* = 2 Hz, H-6). EI-MS: *m/z* 286.

Compound 6 (Luteolin 8-C-β-glucoside), (Orientin): 17 mg, yellow crystals. UV: λ_{max} (nm) (MeOH): 252, 266, 348, (NaOMe): 268, 403, (AlCl₃): 272, 302, 332, 422, (AlCl₃/HCl): 268, 302, 357, 384, (NaOAc): 267, 355, (NaOAc/H₃BO₃): 262, 371. ¹H NMR (DMSO-d₆, 400 MHz): δ ppm 7.52 (1 H, dd, *J* = 2, 8.2 Hz, H-6'), 7.45 (1 H, d, *J* = 2 Hz, H-2'), 6.84 (1 H, d, *J* = 8.2 Hz, H-5'), 6.65 (1 H, s, H-3), 6.22 (1H, s, H-6), 4.64 (1H, d, *J* = 9.5 Hz, H-1'), 3.2- 4.1 (rest of sugar protons). ¹³C NMR (DMSO-d₆, 100 MHz): δ ppm 181.65 (C-4), 166.95 (C-2), 163.85 (C-7), 160.34 (C-5), 156.25 (C-9), 151.2 (C-4'), 146.45 (C-3'), 121.98 (C-1'), 119.25 (C-6'), 115.78 (C-5'), 113.47 (C-2'), 104.58 (C-8), 103.18 (C-10), 101.85 (C-3), 98.54 (C-6), 81.86 (C-5'), 78.75 (C-2'), 73.62 (C-1'), 70.84 (C-3'), 70.65 (C-4'), 61.64 (C-6').

Compound 7 (Luteolin 6-C-β-glucoside) (Isoorientin): 19 mg, yellow crystals. UV: λ_{max} (nm) (MeOH): 245, 267, 345 (NaOMe): 225, 264, 406, (AlCl₃): 225, 262, 365, (AlCl₃/HCl): 263, 282, 296, 358, 365, (NaOAc): 258, 268, 294, 340, 354, (NaOAc/H₃BO₃): 271, 307, 352. ¹H NMR (DMSO-d₆, 270 MHz): δ ppm 7.45 (1H, dd, *J* = 2.5, 8.2 Hz, H-6'), 7.4 (1H, d, *J* = 2.5 Hz, H-2'), 6.9 (1H, d, *J* = 8.2 Hz, H-5'), 6.64 (1H, s, H-3), 6.47 (1H, s, H-8), 4.62 (1H, d, *J* = 9.5 Hz, H-1'), 3.1-4.06 ppm (rest of sugar protons). ¹³C-NMR (DMSO-d₆, 100 MHz): δ ppm 182.45 (C-4), 164.74 (C-7), 164.35 (C-2), 161.62 (C-9), 156.98 (C-5), 150.55 (C-4'), 146.64 (C-3'), 122.18 (C-1'), 119.65 (C-6'), 116.82 (C-5'), 113.94 (C-2'), 109.56 (C-6), 104.25 (C-10), 103.48 (C-3), 94.35 (C-8), 82.15 (C-5'), 79.85 (C-2'), 73.75 (C-1'), 71.48 (C-3'), 70.94 (C-4'), 62.35 (C-6').

Compound 8 (Kaempferol 3-O-α-rhamnoside): 14 mg, yellow powder. ¹H-NMR (CD₃OD, 400 MHz): δ 7.72 (2H, d, *J* = 8 Hz, H-2', 6'), 6.95 (2H, d, *J* = 8 Hz, H-3', 5'), 6.44 (1H, d, *J* = 2 Hz, H-8), 6.28 (1H, d, *J* = 2 Hz, H-6), 5.28 (1H, d, *J* = 2 Hz, H-1'), 0.94 (CH₃, d, *J* = 6 Hz). ¹³C-NMR (CD₃OD, 100 MHz): δ ppm 179.8 (C-4), 166.2 (C-7), 161.8 (C-5), 159.5 (C-4'), 158.2 (C-2), 136.4 (C-9), 132.2 (C-3), 122.9 (C-6'), 116.8 (C-2'), 116.2 (C-3'), 106.1 (C-1'), 103.7 (C-5'), 104.7 (C-10), 100.1 (C-1'), 95.1 (C-8), 94.9 (C-6), 73 (C-5'), 72.4 (C-3'), 72.3 (C-2'), 72.2 for (C-4'), 17.9 (CH₃-rhamnosyl).

Compound 9 (Kaempferol 3-O-β-glucoside): 12 mg, yellow amorphous powder. UV λ_{max} (MeOH): 268, 348, (NaOMe): 272, 324 sh, 398, (AlCl₃): 273, 302 sh, 348, 398, (AlCl₃/HCl): 273, 302 sh, 348, 398 (NaOAc): 274, 312 sh, 367, (NaOAc/H₃BO₃): 268, 344. ¹H-NMR (500 MHz, CD₃OD): δ 8.15 (2H, d, *J* = 9 Hz, H-2', H-6'), 6.95 (2H, d, *J* = 9 Hz, H-3', H-5'), 6.42 (1H, d, *J* = 1.9 Hz, H-8), 6.25 (1H, d, *J* = 1.9 Hz, H-6), 5.32 (1H, d, *J* = 7.8 Hz, H-1'), 3.1-3.9 (5H, m, H-2'', 3'', 4'', 5'', 6'').



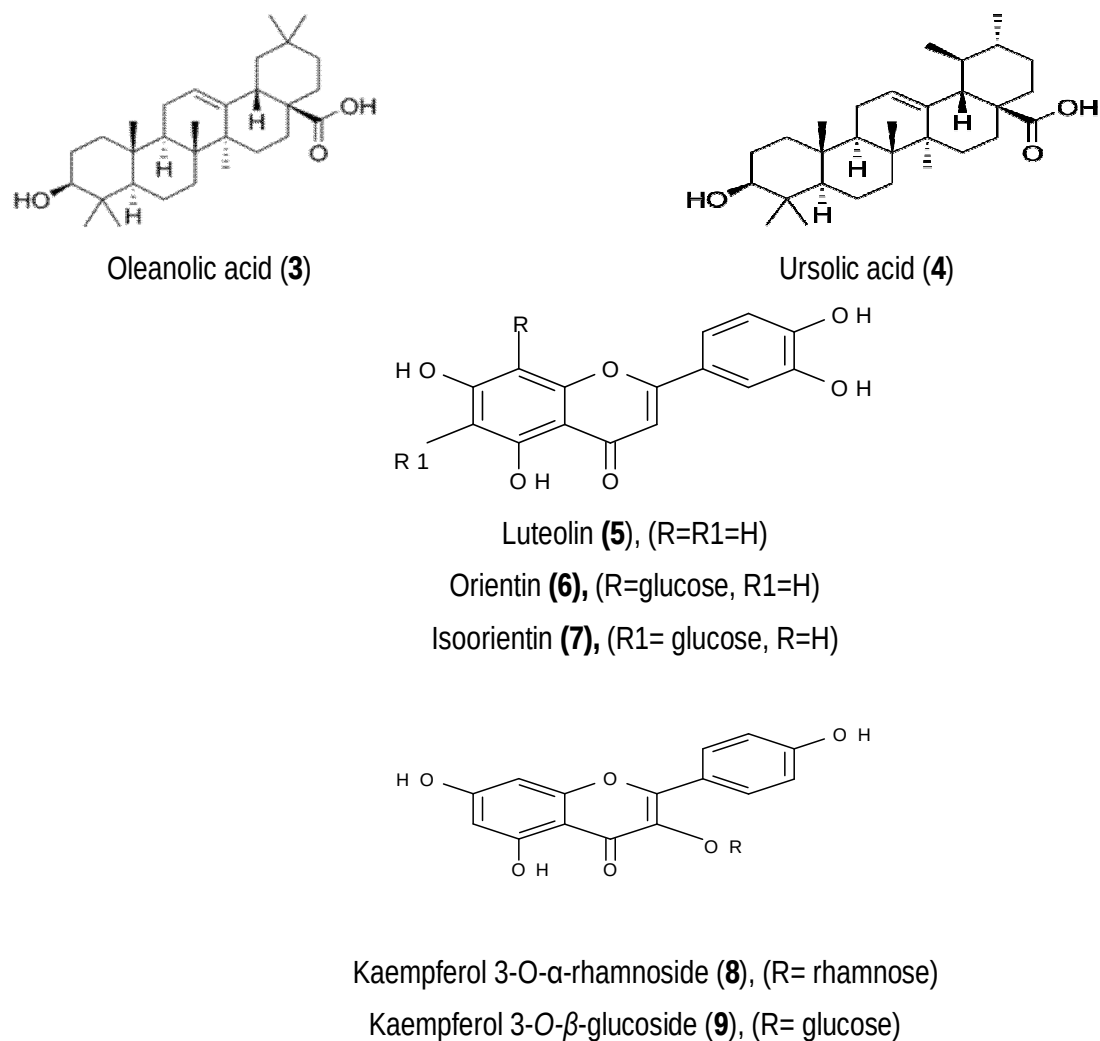


Figure 1. Chemical structures of the compounds isolated of *L. styraciflua* methanol extract

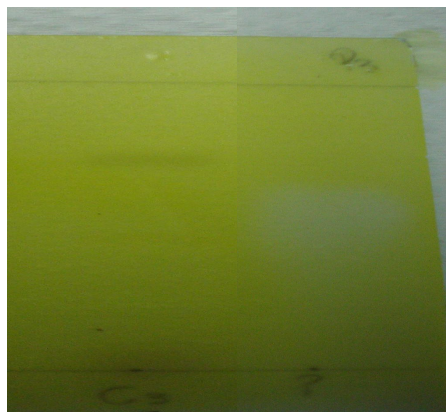


Figure 2. Acetylcholinesterase inhibition of *L. styraciflua* methanol extract (C-3) in TLC and caffeine is used as positive control for acetylcholinesterase inhibitor.

DISCUSSION

Plant extracts are of the most attractive sources of new drugs and have been shown to produce promising results in different pharmacological activities due to the presence of bioactive compounds. Nine compounds were isolated from *L. styraciflua* aerial parts methanol extract and their identification were elucidated by different spectroscopic tools (UV, ^1H -NMR, ^{13}C -NMR, MS). Compound 1 (β -sitosterol) which gave a dark spot under short UV light and changed to violet upon spraying with vanillin-sulphuric acid and heating in an oven at 110°C for 5 minutes. NMR spectral data showed signals very close to β -sitosterol [11]. Compound 2 (lupeol) gave a dark spot under short UV light and changed to pink to violet upon spraying with vanillin-sulphuric acid and heating in an oven at 110°C for 5 min. NMR spectral data showed signals very similar to lupeol [12]. Compound 3 (oleanolic acid) and compound 4 (ursolic acid), both triterpenic acids were detected through spraying with vanillin sulphuric and heating in an oven at 110°C for 5 min and both compounds gave violet spot at visible light, also NMR and MS spectral data are in accordance with published literature [13]. Compound 5 (luteolin) showed a deep purple spot under UV light which changed to yellow with ammonia vapor indicating that a flavone with free 5-OH and 4'-OH [14] and spectral data of compound 5 is very close to that of Owen et al. 2003 [15]. Compound 6 (orientin) and compound 7 (isoorientin) showed a deep purple spot under UV light which changed to yellow with ammonia vapor indicating that they are flavones with free 5-OH and 4'-OH [14]. Complete acid hydrolysis of the two compounds revealed that no sugars were detected meaning that they remained without change in addition to the appearance of an additional spot on the chromatogram (Wessely-Moeser rearrangement between C-6 and C-8), which may be due to acid isomerization [16], indicating that the compounds are mono C-glycosides. Thus, the compounds were subjected to ferric chloride degradation, and co-chromatographed with authentic sugars samples, where glucose was detected. UV spectral data, ^1H NMR and ^{13}C -NMR signals for the two compounds are very similar to the ones reported for orientin and isoorientin, respectively [17, 18]. Compound 8 (kaempferol 3-O- α -rhamnoside) and compound 9 (kaempferol 3-O- β -glucoside) gave a deep purple spot under UV light which changed to yellow with ammonia vapor and spraying with AlCl_3 and complete acid hydrolysis yielded kaempferol as an aglycone, rhamnose and glucose sugar moieties, respectively. The spectral data of both compounds 8, 9 are very similar to that of Amal et al. [19].

Acetylcholinesterase inhibition by methanol extract of *L. styraciflua* aerial parts

The qualitative results of inhibition of enzyme acetylcholinesterase in Thin Layer Chromatography (TLC) showed that the methanol extract the *L. styraciflua* significantly inhibited the enzyme by the appearance yellow backgrounds with white spots for inhibiting compounds were visible after about 5 minutes. This are the results of the first tests, yellow backgrounds with white spots for inhibiting compounds were visible after about 5 minutes for methanol extract of *L. styraciflua* apparently tested positive enzyme inhibition in concentration of 10 mg/mL (Figure 2). The results of acetylcholinesterase inhibition quantitative for methanol extract of *L. styraciflua* that presented strong activity in both tests, the IC_{50} values were determined ($\text{IC}_{50} = 0.07$ mg/mL). The concentration of inhibition 50% (CI_{50}) was tested starting at five different concentrations (1 mg/mL; 0.5 mg/ml; 0.25 mg/mL; 0.125 mg/mL; 0.0625 mg/mL) tested in triplicate, showed that methanol extract of *L. styraciflua* has higher inhibition activity (*L. styraciflua*, $\text{IC}_{50} = 0.07$ mg/mL), in comparison to commonly used drugs neostigmine de ($\text{IC}_{50} = 1.87$ $\mu\text{g/mL}$) and galanthamine ($\text{IC}_{50} = 0.37 \times 10^{-3}$ mg/mL). Galanthamine which is alkaloid considered to be the most effective compound in the treatment of Alzheimer's disease [20]. *L. styraciflua* aerial parts methanol extract seems of interest for further study. Plants that have shown favorable effects in relation to cognitive disorders, including anticholinesterase, anti-inflammatory and antioxidant activities or other relevant

pharmacological activities are potentially of interest to clinical use for Alzheimer's disease [21]. Eighteen medicinal plants of Brazil were screened for inhibitory activity on AChE, the results show that various plants are very interesting for further isolation of acetylcholinesterase inhibitors, which are widely used in the treatment of Alzheimer's disease, galanthamine, an alkaloid from plants of the Amaryllidaceae family, is a selective reversible long-acting and competitive acetylcholinesterase inhibitor (AChEI). The extract is considered to be more effective in the treatment of Alzheimer's disease (AD) and to have fewer limitations than physostigmine and tacrine are relevant in terms of searching for novel formulations or compounds for AD treatment [20]. This is the result of the first tests, yellow backgrounds with white spots for inhibiting extract was visible after about 5 minutes and so *L. styraciflua* aerial parts methanol extract apparently tested positive enzyme inhibition in concentration of 10 mg/mL. The activity of the methanol extract of *L. styraciflua* may be explained by the presence of carbohydrates, triterpenes, flavonoids and tannins and also for the isolated bioactive compounds where some of the isolated compounds (kaempferol sugars) showed a significant acetylcholinesterase inhibition [22]. Also many plants as *Sophora flavescens* showed a significant acetylcholinesterase inhibition and this activity is due to prenylated flavonoid, 8-lavandulylkaempferol which exhibited significant inhibitory effects with IC_{50} values of 7.10 and $8.11\mu M$ for butyrylcholinesterase and acetylcholinesterase [23], also Inhibition of Acetyl Cholinesterase by *Indigofera* species extracts was due to the pote

CONCLUSION

The methanol extract of *L. styraciflua* apparently tested positive acetylcholinesterase enzyme inhibition, it has shown ($IC_{50} = 0.07$ mg/mL). This activity may be due to the presence of bioactive compounds as (β -sitosterol, lupeol, oleanolic acid, ursolic acid, luteolin, luteolin 8-C- β -glucoside (orientin), luteolin 6-C- β -glucoside (isoorientin), kaempferol 3-O- α -rhamnoside and kaempferol 3-O- β -glucoside isolated from the extract. These active compounds have a similar activity to neostigmine ($IC_{50} = 1.87\mu g/mL$) and galanthamine ($IC_{50} = 0.37 \times 10^{-3}$ mg/mL). These results prove that the extract can act as acetylcholinesterase inhibitor and can be widely used in the treatment of Alzheimer's disease.

CONFLICT OF INTEREST

There is no conflict of interest associated with the authors of this paper.

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